A BASIC CHLOROPHYLL–PROTEIN COMPLEX

M. KRISHNAN and A. GNANAM
School of Biological Sciences, Madurai University, Madurai 625 021, India

Received 2 October 1978

1. Introduction

The presence of two pigment–protein complexes using SDS–polyacrylamide gel electrophoresis was reported [1]: one of lower mobility, the P-700 chlorophyll *a*–protein complex; the other of faster mobility, the light-harvesting chlorophyll *a*/*b*–protein complex. Additional pigment–protein complexes have been reported with improvements in the experimental conditions and techniques [2–6]. The presence of an additional pigment–protein complex was reported [3] called the dimer of complex IIc. Two additional pigment–protein complexes were described [4] of which one was shown to be the dimer of complex IIc, probably that in [3], but did not characterize the other additional pigment–protein complex because of the low quantity. An additional complex was reported [6] called complex A which was shown to contain the reaction centre of photosystem II, based on indirect evidence. We describe here a new pigment–protein complex of mol. wt 15 000 and discuss its possible relationship to the other pigment–protein complexes.

2. Materials and methods

The thylakoid membranes from the mesophyll chloroplasts of *Sorghum vulgare* were isolated using Tris–HCl buffer (pH 6.8) 0.0625 M without osmoticum (sample buffer). The chloroplast membrane suspension was diluted with the sample buffer to 1 mg/ml chlorophyll equivalent. Enough SDS was added from 10% stock solution to the chloroplast membrane suspension to bring the ratio of chl:SDS to 1:5 and the suspension was homogenized as in [6] using Teflon hand homogenizer. All the steps leading to the loading of the sample were completed in 15 min. Electrophoresis was done immediately after solubilization of the chloroplast membranes at 4°C in total darkness for 5 h. Special gel tubes of 25 mm diam. were used to get the required amounts of the complexes for further characterization. Gel tubes of 6–55 mm diam. were tried and it was found that 25 mm diam. tubes were the most convenient for use. A 10% polyacrylamide separation gel and 4% stacking gel were used for electrophoresis [7]. The pigment–protein complex was extracted from the gel in 80% acetone and the absorption spectrum was monitored using Gilford Spectrophotometer 250. Polypeptide analysis of pigment–protein complex was performed by overnight extraction of the finely ground gel in the minimal volume of the sample buffer in slab gels. The gel was scanned using ISCO gel scanner UA-5.

3. Results

The electrophoretic pattern of the thylakoid membranes solubilized with SDS showed 10 green bands including the one with the fastest mobility, viz., the free chlorophyll complexed with SDS (fig.1). The bands were designated in the order of their increasing mobility as Ia, Ib, I, IIa, B, IIb, A, IIc, IId and FP, of which the pigment–protein complexes I, IIa, IIb, A and IIc have been reported [1–6]. The additional pigment–protein complexes reported have been located between the complexes I and IIc. Henriques (personal communication) observed a pigment–protein complex of mobility lower than that of the complex I. We report the presence of 4 new pigment–protein complexes Ia, Ib, B and IId of which we were

 Elsevier/North-Holland Biomedical Press

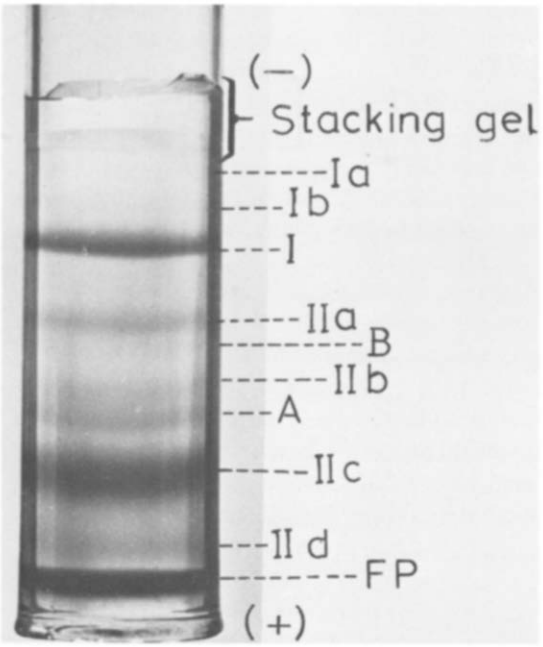

Fig.1a

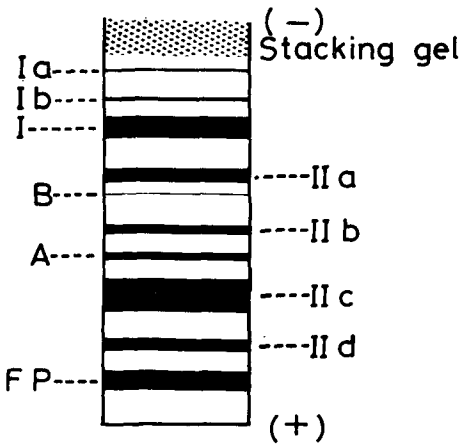

Fig.1b

Fig.1. (a) Electrophoretogram of SDS-solubilised thylakoid membranes before staining showing 10 chlorophyll-containing regions. (b) Diagramatic representation.

able to characterize only the complex IId. The characterization of the complexes Ia, Ib and B was not possible since enough quantities of these complexes could not be obtained even in the special gel tubes.

The absorption spectrum of the complex IId shows an A_{669} max and a shoulder at A_{648} (fig.2). The polypeptide analysis of the complex IId shows the presence of only 1 polypeptide of mol. wt 15 000 (fig.3).

3.1. *Behaviour of the complexes during electrophoresis*

The complex IIa when re-electrophoresed dissociated into the complexes IIb, IIc and IId, retaining a faint band of its own in the gel. Instead of extracting the complexes from the gel and re-electrophoresing, the gel portion containing the complex was cut and placed on the separation gel. The stacking gel was not used for re-electrophoresing the complexes. On re-electrophoresis, it was possible to obtain from the complex IIb, a small quantity of the same and considerable quantities of the complexes IIc and IId. Likewise, we obtained the complexes IIc and IId from the complex IIc on re-electrophoresis. We could not observe the complexes A and B after re-electrophoresing the complexes IIa and IIb. Also, it was not possible to obtain the complexes of faster mobility from complex I on re-electrophoresis.

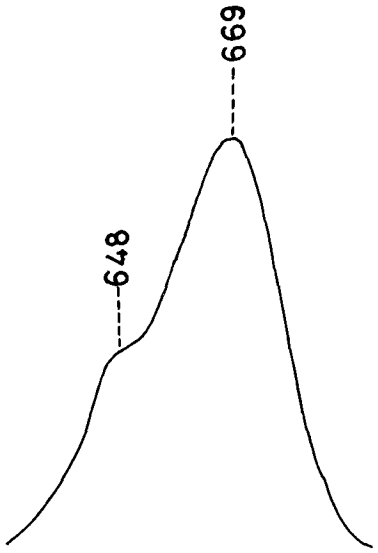

Fig.2. Room temperature absorption spectrum of the complex IId after extraction from the gel.

Fig.3. Densitometer tracing at 546 nm of the polypeptide from the complex IId, after staining with 0.1% amido black.

4. Discussion

We report here the presence of 9 complexes excluding the free pigment complexed with SDS. As many complexes could not be obtained earlier probably due to the lack of a controlled method of chloroplast membrane solubilisation with SDS. The technique of controlling solubilisation was provided [6] using a Teflon hand homogenizer. We found 3 gentle strokes with the hand homogenizer very effective for gentle solubilisation, which we believe, was essential for obtaining 9 pigment–protein complexes. In addition to this, keeping the temperature very low (0–4°C) and running electrophoresis for a longer time in total darkness were found to be the other effective factors.

There is general agreement that complex IIc is the basic complex. It was shown [6] that the basic complex was IIc and that IIb and IIa are the dimer and trimer of complex IIc, respectively. The complexes IIa and IIb may also be the oligomers of the complex IIc [6].

Based on the results obtained by re-electrophoresing the complexes IIa, IIb and IIc we suggest that the monomer of the above complexes is IId and not IIc as reported. The possible existence of a structural protein of mol. wt 10 000, basic to all the protein components of the thylakoid membranes was reported [8] and the observed heterogeneity of the chloroplast membrane proteins in SDS–polyacrylamide gel electrophoresis was ascribed to polymerization of the basic unit. Our experimental data suggest that at least the complexes IIa, IIb and IIc are the oligomers of the basic pigment–protein complex IId. On the basis of the report [8] on the basic unit we extrapolate our observations to advocate that the protein moiety of complex IId may be the basic unit to all the complexes of the lower mobility. However, this remains to be supported by further experiments.

References

[1] Thornber, J. P. (1975) Ann. Rev. Plant Physiol. 26, 127–158.
[2] Herrmann, F. and Meister, A. (1975) Photosynthetica 6, 177–182.
[3] Hiller, R. G., Genge, S. and Pilger, D. (1974) Plant Sci. Lett. 2, 239–242.
[4] Remy, R., Hoarau, J. and Leclerc, J. C. (1977) Photochem. Photobiol. 26, 151–158.
[5] Hayden, D. B. and Hopkins, W. G. (1977) Can. J. Bot. 55, 2525–2529.
[6] Henriques, F. and Roderic Park (1978) Biochem. Biophys. Res. Commun. 81, 1113–1118.
[7] Laemmli, U. K. (1970) Nature 227, 680–685.
[8] Lagoutte, B. and Duranton, J. (1976) Biochim. Biophys. Acta 427, 141–152.